failed to affect the channel's total protein level and membrane trafficking. Our data suggest that 14-3-3 is a novel modulator of rEag1 channels in the brain.

675-Pos Board B461
Voltage-Dependent Potassium Channels Kv1.3 and Kv1.5 in Human Cancer
Joanna Bielanska[1], Javier Hernandez-Losa[2], Mireia Perez-Verdaguer[1], Teresa Moline[2], Rosa Somoza[2], Santiago Ramon y Cajal[2], Enric Condom[3], Joan Carles Ferreres[2], **Antonio Felipe**[1].
[1]University of Barcelona, Barcelona, Spain, [2]Hospital Universitari Vall d'Hebron, Barcelona, Spain, [3]Hospital Universitari de Bellvitge-IDIBELL, Barcelona, Spain.
Membrane ion channels participate in cancerous processes such as proliferation, migration and invasion, which contribute to metastasis. Increasing evidence indicates that voltage-dependent K+ (Kv) channels are involved in the proliferation of many types of cells, including tumor cells. Kv channels have generated immense interest as a promising tool for developing new antitumor therapies. Therefore, the identification of potential biomarkers and therapeutic targets in specific cancers is an important prerequisite for the treatment. Since Kv1.3 and Kv1.5 are involved in the proliferation of many mammalian cells, we aimed to study the expression of Kv1.3 and Kv1.5 in a plethora of human cancers. Thus, tissue from breast, stomach, kidney, bladder, lung, skin, colon, ovary, pancreas, brain, lymph node, skeletal muscle and some of their malignant counterparts have been analyzed. Whereas Kv1.3 expression was either decreased or did not change in most tumors, Kv1.5 was overexpressed. However, the presence of Kv1.3 was mostly associated with inflammatory lymphoplasmocytic cells. Independent of the suitability of individual channels as therapeutic targets, the identification of a Kv phenotype from tumor specimens could have a diagnostic value of its own. Our results demonstrate that Kv1.5, and to some extent Kv1.3, are aberrantly expressed in a number of human cancers. These channels could serve both as novel markers of the metastatic phenotype and as potential new therapeutic targets. The concept of Kv channels as therapeutic targets or prognostic biomarkers attracts increasing interest and warrants further investigation.
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676-Pos Board B462
A Model of β1-Adrenergic Regulation of L-Type Ca^{2+} Current and Ryanodine Receptors in Mouse Ventricular Myocytes
Maria V. Shorina, **Vladimir E. Bondarenko**.
Georgia State University, Atlanta, GA, USA.
Mathematical modeling protein signaling networks is one of the most rapidly developing fields of science. This includes creation and investigation of the models for protein signaling systems in the heart cells. β_1-adrenergic signaling system is one of the most important systems in cardiac myocytes. We developed an experimentally-based mathematical models of β_1-adrenergic regulation of L-type Ca^{2+} current and ryanodine receptors in mouse ventricular myocytes. The model includes several modules and describes signal transduction in the cells. In the model, β_1-adrenergic receptors (β_1-ARs) are stimulated by application of β_1-adrenergic agonist isoproterenol. Activation of β_1-ARs in turn activates G_s proteins, $G_{s\alpha}$ subunit of which subsequently stimulates cyclic AMP (cAMP) synthesis by adenylyl cyclases. cAMP further activates protein kinase A (PKA) holoenzyme and dissociates PKA catalytic subunit from regulatory subunit. cAMP is degraded by phosphodiesterases (PDEs) that can be inhibited by 3-isobutyl-1-methylxanthine (IBMX). Inhibition of PDEs by IBMX leads to an increase in cAMP level and more profound stimulation of PKA. PKA is also regulated by heat-stable protein kinase inhibitor. β_1-ARs are desensitized by β-adrenergic receptor kinase-1 and by catalytic subunit of PKA that creates negative feedback for this signaling pathway. PKA phosphorylates L-type Ca^{2+} channel, which results in an increase of channel's opening probability and current amplitude. Phosphorylation is removed by protein phosphatases 1 and 2A. We developed a Markov model for L-type Ca^{2+} channel that consists of two parallel activation-inactivation pathways for non-phosphorylated and phosphorylated states. There are also transitions between corresponding non-phosphorylated and phosphorylated states in the model (closed, open, or inactivated states). Similar approach was applied to the Markov model development for ryanodine receptors. Both models reproduced experimentally observed behavior of the channels.

677-Pos Board B463
Modulation of $Ca_v2.2$ Channels via Activation of Human $GABA_B$ Receptors Expressed in HEK293 Cells by Analgesic α-Conotoxins
Thuan G. Huynh[1,2], Geza Berecki[1], **David J. Adams**[1].
[1]RMIT University, Melbourne, Australia, [2]University of Queensland, Brisbane, Australia.
Numerous G protein-coupled receptors, including the $GABA_B$ receptor ($GABA_BR$), provide negative feedback to modulate the activity of neuronal (N)-type voltage-gated calcium channels ($Ca_v2.2$), which play a critical role in nociception. We have previously shown that analgesic α-conotoxins indirectly inhibit $Ca_v2.2$ channels via $GABA_BR$ activation in mammalian dorsal root ganglion neurons (1). We reconstituted $GABA_BR$-mediated modulation of stably expressed $Ca_V2.2$ channels (α1, α2δ and β3) in HEK293 cells, co-transfected with cDNAs of cloned human $GABA_BR$ subunits. $GABA_BR$ expression was demonstrated using receptors labelled with fluorescent antibodies against the epitope tags of $GABA_{B1}$ and $GABA_{B2}$ subunits. Modulation of $Ca_v2.2$ by the agonists GABA and baclofen, and the α-conotoxins Vc1.1 and RgIA was studied using the whole-cell recording configuration of the patch clamp technique. Voltage-dependent Ba^{2+} currents were inhibited by baclofen, GABA and α-conotoxins Vc1.1 and RgIA in HEK293 cells transfected with $Ca_v2.2$ and $GABA_BRs$ but not in cells transfected with $Ca_v2.2$ alone. In the presence of the $GABA_BR$, the biophysical properties of the $Ca_v2.2$ channels, including the current-voltage relationship, and activation and steady-state inactivation curves, were unchanged. The concentration-response relationships obtained for inhibition of $Ca_v2.2$ by baclofen, GABA, RgIA, Vc1.1, and cyclized Vc1.1 resulted in half-maximal inhibitory concentrations (IC_{50}) of 3 μM, 68 nM, 130 nM, 120 nM and 10 nM, respectively. The inhibition by baclofen and GABA could be reversed by a depolarizing prepulse to +80 mV, whereas the effect of the α-conotoxins was slower and unaffected by a prepulse, suggesting the involvement of a voltage-independent pathway. Taken together, HEK293 cells provide a suitable expression system to study $GABA_BR$ modulation of $Ca_v2.2$ channels and confirm the role of $GABA_BRs$ in mediating the effects of analgesic α-conotoxins.
1. Callaghan et al. (2008) *J. Neurosci*. **28:**10943-51.

678-Pos Board B464
RGS Proteins Maintain Robustness of GPCR-GIRK Coupling
Huai-hu Chuang[1], Alexander Y. Chuang[2].
[1]Cornell University, ITHACA, NY, USA, [2]Lowell High School, San Francisco, CA, USA.
Regulators of G-protein signaling (RGS) are GTPase activating proteins (GAP) that reduce response amplitudes of activated G-protein coupled receptors (GPCRs). We discovered that, although RGS proteins drastically accelerate kinetics of GPCR-coupled K+ currents (GIRK), they actually increased amplitudes of inhibitory neurotransmitter-evoked GIRK currents. The RGS-Box domain alone is sufficient for stimulation of transmitter activation of K+ currents, but its membrane association enhances the efficiency of stimulation. Moreover, RGS4 mutants with compromised GAP activity still augment GPCR-GIRK coupling. Among the pertussis toxin sensitive G-proteins, we found that RGS4 selectively stimulates G alpha-o to maintain robustness of GPCR-GIRK coupling. Opposing actions of RGS proteins thus both stimulate and inhibit G-proteins to modulate ultimate amplitudes of transmitter-induced GIRK currents and to differentiate signal intensity coupled to various G-protein isoforms.

679-Pos Board B465
Active Site Hydration and Water Diffusion in Cytochrome P450Cam: A Highly Dynamic Process
Yinglong Miao[1,2], Jerome Baudry[1,2].
[1]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee-Knoxville, Knoxville, TN, USA, [2]University of Tennessee/Oak Ridge National Laboratory Center for Molecular Biophysics, Oak Ridge, TN, USA.
Cytochrome P450s are essential hemoprotein monooxygenases that catalyze a variety of biochemical processes including drug metabolism, lipid and steroid biosynthesis, and degradation of pollutants. As an enzyme from Pseudomonas putida that catalyzes the regio- and stereo-specific hydroxylation of camphor, cytochrome P450cam has long served as a model system for studying P450s. Water molecules are known to play an important role in the enzymatic activity and must be able to enter and exit the active site of P450cam. Here long-timescale molecular dynamics (MD) simulations (300 ns) are performed on both the apo- and camphor-bound P450cam. Water diffusion into and out of

protein active site is observed without biased sampling methods. During the course of MD simulation, an average of 6.4 water molecules is observed in the camphor-binding region of the apo form and zero water molecules in the binding site of the substrate-bound form, in agreement with the number of water molecules observed in their crystal structures. However, as many as 12 water molecules can be present at a given time in the camphor-binding region of the active site in apo-P450cam, revealing a highly dynamic process for hydration of the protein active site with water molecules exchanging rapidly with the bulk solvent. Water molecules are also found to frequently exchange locations inside the active site, preferentially clustering in regions surrounding the water molecules observed in the crystal structure. Potential of mean force calculations identify thermodynamically favored trans-protein pathways for the diffusion of water molecules between protein active site and the bulk solvent. Binding of camphor in the active site modifies the free energy landscape of P450cam channels toward favoring the diffusion of water molecules out of protein active site.

680-Pos Board B466

Computational Systems Analysis of Glucose Sensitivity, Electrical Activity and Glucagon Secretion in Pancreatic Alpha-Cells

Leonid Fridlyand, Louis H. Philipson.
University of Chicago, Chicago, IL, USA.

Glucagon, a 29-amino acid hormone secreted from the alpha-cells of the islets of the endocrine pancreas, is critical for blood glucose homeostasis by promoting liver production of glucose in hypoglycemia. A clearly defined, testable model for alpha cell glucagon secretion is lacking. Alpha-cells respond to rising blood glucose by increasing oxidative metabolism, closing ATP-sensitive K+ channels and the resulting depolarization of the plasma membrane leads to decreased glucagon secretion. This mechanism of glucose sensing involves the coupling of electrophysiological, cytoplasmic and mitochondrial processes. Here we describe a computational systems analysis of pancreatic alpha-cells including metabolic processes , electrical activity and Ca2+ dynamics in the regulation of glucagon secretion. This mathematical model of alpha-cell sensitivity to glucose is based on a previous model for pancreatic beta-cells. We also formulated a Hodgkin-Huxley-type ionic model for action potentials in alpha-cells that incorporates voltage-gated Ca2+, K+, Na+ and Cl- currents. The metabolic and ionic models are coupled to the equations describing intracellular Ca2+ homeostasis and glucagon secretion that depends on an activation of specific voltage-gated Ca2+ channels. This model simulates the behavior of alpha-cell action potentials under a wide range of experimental conditions, including block of action potential firing at increased glucose levels or due to channel blockade. Paracrine regulation of glucagon secretion can be modeled following the effects of gamma aminobutyric acid or somatostatin secreted by other islet cells on corresponding alpha-cell ion channels and metabolism. This computational systems analysis aids in providing a more complete understanding of the complex process of alpha-cell glucose sensing and the pathways of treatment of diabetes.

681-Pos Board B467

Molding the Business End of Neurotoxins by Diversifying Evolution

Noam Zilberberg, Adi Kozminsky-Atias.
Ben Gurion University, Beer Sheva, Israel.

A diverse range of organisms utilize neurotoxins that target specific ion channels and modulate their activity. Typically, toxins are clustered into several multigene families, providing an organism with the upper hand in the never-ending predator-prey arms race. Several gene families, including those encoding certain neurotoxins, have been subject to diversifying selection forces, resulting in rapid gene evolution. Here, we sought a spatial pattern in the distribution of both diversifying and purifying selection forces common to neurotoxin gene families. Utilizing the Mechanistic Empirical Combination model, we analyzed various toxin families from different phyla affecting various receptors and relying on diverse modes of action. By this approach, we were able to detect clear correlations between the pharmacological surface of a toxin and rapidly evolving domains, rich in positively selected residues. On the other hand, patches of negatively selected residues were restricted to the non-toxic face of the molecule and most likely help in stabilizing the tertiary structure of the toxin. We thus propose a mutual evolutionary strategy of venomous animals in which adaptive molecular evolution is directed towards the toxin active surface. Furthermore, we propose that the binding domains of unstudied toxins could be readily predicted using evolutionary considerations.

682-Pos Board B468

Cooperative Regulation of Slack Channel by Na^+, Cl^- and PIP2

Zhe Zhang, Qiongyao Tang, Diomedes E. Logothetis.
Virginia Commonwealth University, Richmond, VA, USA.

Slack (or Slo2.2) channel activity is regulated by Na+ and Cl- ions. The Na+ coordination site of Slack channels bears similarity to the analogous site in Kir3 channels. Here, we show that the activity of Slack channels, like BK (or Slo1) and Slo3 channels, is also dependent on PIP2. We next ask: How are these multiple positive cooperative factors interact with each other to regulate Slack channel activity? We first examined the relationship of Na+ and PIP2 regulation. Unlike Kir3 channels, the D818N mutation, which decreases Na+ sensitivity greatly, showed no effect on PIP¬2 sensitivity. Manipulations that weaken channel-PIP2 interactions in Kir2.1 channels induce residence into sub-conductance states (Xie et al., 2008, J. Physiol. 586:1833). Unitary Slack channel activity rarely visited sub-conductance levels in the absence of Cl-. Yet, in the presence of Cl-, the Slack single channel conductance was shifted from 145 pS to 80~100 pS, while the open probability of Slack channels was increased by 2-2.5 fold due to the cooperative regulation of Cl- with Na+. We are in the process of screening for potential Cl- binding site(s) in the cytoplasmic domain of Slack channels, utilizing a strategy similar to the one that enabled us to identify the D818 Na+ coordination site. We hypothesize a close cooperative relationship between PIP2 and Cl- regulation of Slack activity. We are working on investigating this cooperativity and developing a suitable model to explain this complex regulatory mechanism.

683-Pos Board B469

Structure and Elasticity of Genistein and Daidzein in Lipid Membranes using X-Ray Scattering and MD Simulations

Mohit Raghunathan[1], Yuriy Zubovski[1], Richard M. Venable[2], Richard W. Pastor[2], John F. Nagle[1], **Stephanie Tristram-Nagle**[1].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]National Heart, Lung and Blood Institute, Bethesda, MD, USA.

This work reports the effects of bioflavinoids genistein and daidzein on lipid bilayers as determined by volume measurements, X-ray scattering and molecular dynamics simulations. Upon adding 20 mole% genistein or daidzein to DOPC membranes, experimental and simulated volumes are found to be in outstanding agreement. Both bioflavinoids insert into the hydrocarbon region near the carbonyls of DOPC and also DiphytanoylPC, ~12 Å from the bilayer center. In DOPC, both experiments and MD simulations show the area/unit cell equals 83 $Å^2$ for 20 mole% genistein and 80 $Å^2$ for 14 mole% daidzein (its maximum solubility in oriented samples). Both bioflavinoids thin DOPC and DPhyPC membranes proportional to their solubility. The long axes of both bioflavinoids are oriented nearly parallel to the plane of the bilayer with their carbonyl groups preferentially pointed towards the proximal surface. X-ray diffuse scattering reveals that both bioflavinoids modestly reduce K_C, the bending modulus, daidzein slightly more than genistein. MD simulations determine that both bioflavinoids reduce K_A, the area compressibility modulus, daidzein slightly more than genistein. These elasticity results are in general agreement with the hypothesis that bioflavinoids increase membrane flexibility, thereby overcoming hydrophobic mismatch, and increase gramicidin (gA) lifetimes[1]. However, the small structural and elasticity differences between genistein and daidzein cannot account for genistein's having twice the effect of daidzein in increasing gA channel lifetimes; that is apparently due to the greater solubility of genistein in membranes when equal amounts are added in gA experiments.
[1]Hwang, Koeppe & Andersen, Biochemistry 42:13464(2003).

684-Pos Board B470

Electrophysiological and Gene Expression Profiling at Single Cell Level through an Improved Whole Cell Patch Clamp Quantitative Real-Time PCR Technique

Ken Veys, **Alain J. Labro**, Dirk J. Snyders.
University of Antwerp, Antwerp, Belgium.

Cellular excitability andaction potential generation originates from a concerted action of different ion channels. The molecular diversity of channels (over 100 different genes) underlies the vast diversity in the CNS and even a specific type of neurons may display large differences in channel expression. Patch-clamp is a powerful technique to study the electrophysiology of excitability at the single cell level allowing to explore cell-to-cell variability. At present only few attempts have been made to link electrophysiological profiling to mRNA transcript levels and most suffered from experimental noise making correlations virtually impossible. Here we describe a significant refinement to the technique that combines patch-clamp analysis with quantitative real-time (qRT) PCR at the single cell level. Hereto the process was optimized such that the expression of a housekeeping gene could be used to normalize for cell-to-cell variability in mRNA isolation as this step relied on capturing most of the cytosol into the patch-pipette. However, the total amount of mRNA obtained from a single neuron remains the limiting factor to have enough cDNA yield for a valid qRT-PCR. This was resolved by designing an RNA amplification step and the technique was validated on a stable Ltk- cell line expressing the Kv2.1 channel. Current density and Kv2.1 transcript quantity displayed a nice correlation ($R^2 = 0.9$) when the qRT-PCR essay was done in twofold and the data